Yield, transpiration efficiency, and water use variations and their relationships in the sorghum reference collection

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Abstract. Sorghum is well adapted to water limited conditions, but the traits responsible for this enhanced adaptation under drought conditions remain unclear. In this study, yield, transpiration efficiency and water extraction were assessed in 149 germplasm entries from the sorghum reference set (plus three control cultivars) using a lysimetric system under terminal water stress and fully irrigated conditions outdoors. A ten-fold range for grain yield and harvest index (HI), two-fold range for transpiration efficiency (TE) and a 1.25 fold variation for water extraction were observed under terminal water stress conditions. Transpiration efficiency and water extraction under water stress related poorly to that under fully irrigated conditions, reflecting a large genotype-by-water treatment interaction. Under drought stress, total water extraction varied by about 3 L plant^{-1} among germplasm. Entries from the Durra race had highest water extraction capacity, whereas Caudatum-Bicolor and Caudatum-Durra intermediate races had poor water extraction. Durra, Caudatum and Caudatum-Guinea races had highest TE, whereas the Guinea race had the lowest. Although yield was closely related to HI, at any level of HI there were substantial yield differences that remained unexplained, and these residual yield variations were closely related to transpiration efficiency (R^2 = 0.60). Similarly, substantial yield variations that were still not explained by HI or TE were closely related to the total water extracted under water stress (R^2 = 0.35). A multilinear regression analysis confirmed these results and showed the importance of water extraction during grain filling. Therefore, next to HI, the yield differences under terminal drought in

sorghum were driven by TE, and then next by water extraction. The large genetic variation for TE and water extraction offer great breeding opportunities and, in particular, highlight the Durra race as a critical source of variation.

Additional keywords: Roots, germplasm reference set, water uptake profile, pre-anthesis water use.

Introduction

Water deficit is the most important abiotic stress and significantly limits crop production globally, particularly in the Semi-Arid Tropics (SAT). There are different "patterns" of water stresses depending on the timing, the intensity, and the duration of drought stress (Serraj *et al.* 2005). In the SAT, where the length of the cropping period is limited, sorghum often faces a terminal drought, caused by the cessation of rain toward the end of the rainy season. This is particularly the case for post-rainy (*rabi*) sorghum in India, which is sown at the end of the rainy season to take advantage of the moisture accumulated in the soil profile. Successful crops under terminal drought are those having increased water availability and accessibility during grain filling (Vadez *et al.* 2007a). Possible options for increasing water availability post-anthesis are to: (i) manage the soil moisture profile in a way that leaves water available for grain filling, including strategies to minimize water use before anthesis (Kholova *et al.* 2010a, 2010b) or strategies to enhance transpiration efficiency; (ii) develop a deeper and/or more profuse rooting system to access extra water from the soil profile.

Having higher transpiration efficiency (TE, in g biomass kg^{-1} water transpired) could contribute to a slower rate of soil moisture depletion. Genotypic differences for TE have been reported in sorghum under well watered conditions (Hammer *et al.* 1997; Xin *et al.* 2009). Few studies have looked at TE under both fully irrigated and water stress conditions (Donatelli *et al.* 1992; Balota *et al.* 2008), with only a limited range of germplasm being assessed. Also, except Balota *et al*. (2008), TE has been measured over relatively limited periods of time. So it is important to assess genetic variation for TE

59 over an entire crop cycle and to determine whether there are large genotype-by-water
60 regime interactions for TE. We used this approach to assess a large and diverse set of
61 germplasm lines from the sorghum reference set (Ramu *et al.* manuscript in preparation).

62 Rooting traits have been reported as potentially important for drought adaptation in
63 sorghum (Bhan *et al.* 1973, Mayaki *et al.* 1976, Blum *et al.* 1977; Jordan *et al.* 1979)
64 based on studies involving a limited number of genotypes. In one study, the roots of a
65 drought tolerant sorghum line grew at least 40 cm deeper than a drought sensitive one
66 (Salih *et al.* 1999). Yet, root measurements are time-consuming and prone to error
67 (Vadez et al., 2007a), and the range of genotypic variation for the capacity to extract
68 water from a soil profile remains unknown. This is critical information to gather since
69 recent simulation and experimental work in wheat shows that each millimeter of water
70 extraction during the grain filling period contributes to an additional 55 kg ha^{-1} grain
71 yield (Manschadi *et al.* 2006) and 59 kg ha^{-1} grain yield (Kirkegaard *et al.* 2007),
72 respectively. It was also shown that the total water extraction did not differ between the
73 tolerant and the sensitive wheat genotype, but the tolerant line used less water before
74 anthesis and more after anthesis than the sensitive line (Manschadi *et al.* 2006). Here,
75 similar hypotheses are developed in sorghum to assess differences in the total water
76 extraction and the proportion of water being used during the post-anthesis period to
77 service grain filling (Hammer 2006).

78 Passioura's equation (1977) (Y = WU x TE x HI, with Y, WU, TE, and HI standing for
79 yield, water used, transpiration efficiency and harvest index) has been widely used to
80 guide the search for traits contributing to drought adaptation. However, since there was
81 no method to evaluate all components on the same plants with an equal degree of
82 precision, the use of that equation was generally limited to only single components,
83 regardless of the relative importance of other components. For example, the past twenty
84 years of drought research in groundnut has focused on water use efficiency (Hubick *et al.*
85 1986; Wright *et al.* 1994; Nageswara Rao *et al.* 2001; Udaykumar *et al.* 1998
86 Krishnamurthy *et al.* 2007), often relying on surrogates to estimate trait value. Similarly,
87 rooting traits have been used as surrogates for water extraction (the WU component)
88 (reviewed in Vadez *et al.* 2007a). Whether high TE relates to low water use (Blum *et al.*
89 2005) or not (Peng and Krieg 1992) is still a matter of debate. Also, it is possible that one

of the components of the equation may have, under specific conditions, a greater bearing on yield, thereby obscuring the true contribution of the other components to yield. Here, a method is used (Vadez *et al.* 2008; Ratnakumar *et al.* 2009) to precisely assess all components of Passioura's equation on same plant and test their relationships using a large set of germplasm.

Sorghum is among the most adapted crops to dryland farming. Yet there is considerable genetic diversity available for adaptation to water deficit (Crasta *et al.* 1999; Harris *et al.* 2007). Recently, a mini core collection of 242 accessions of sorghum germplasm lines representing global diversity in core and entire collections has been developed using data on 21 phenotypic traits (Upadhyaya *et al.* 2009). More recently, a reference set collection based on data from 41 SSR markers consisting of 384 entries has been developed (Ramu *et al*. manuscript in preparation). We assess variation in the traits described above in a sub-set of the reference set of sorghum which was chosen to limit variation in their time to flowering.

The overall objective of the present study was to assess variation in the sorghum reference collection for traits related to plant water use and hypothesized to be closely related to crop adaptation under terminal drought. We specifically assessed: (i) the genotypic differences in water extraction and the pattern of water use before and after anthesis; (ii) the genotypic variation in transpiration efficiency; (iii) the range of water regime-by-genotype interaction for these traits; (iv) the contribution of these traits to grain yield under terminal drought, and (v) possible association between specific sorghum races and values of the traits assessed.

Materials and methods

Soil filling and growth conditions of the lysimeters

Plants were grown in lysimeters, i.e. PVC tubes of 25 cm diameter and 2.0 m length, filled with Alfisol in outdoor conditions, with possibility to cover them with a shelter in case of rain. A PVC end plate was placed on top of four screws at the bottom of the cylinders, 3cm from the very bottom, to prevent the soil from seeping through. The

endplate did not fit the cylinder tightly and allowed water drainage, although drainage was prevented when lysimeter weighing started (see below). Tubes were filled with Alfisol collected from the ICRISAT farm and sieved to particles smaller than 1 cm. This allowed the bulk density of the soil profile to be set at approximately 1.35 g cm^{-3}, a standard value for Alfisols. Cylinders were filled with soil in three increments of 40 kg of dry soil. After addition of each 40 kg increment, the soil level in several cylinders was checked to ensure they were similar in all tubes. Then each 40 kg of soil added was watered. A previous assessment of the water needed to fill the profile before drainage determined that the soil water holding capacity of the Alfisol was approximately 20%. Therefore, 8 L of water were added to each 40 kg increment of soil. After adding/watering 40 kg of soil three times, an additional 15 kg of dry soil was added to each cylinder and watered with 3 L. At that stage, the cylinders were almost filled to the desired level, i.e. approximately 5 cm from the top. A top-up using dry soil was done to ensure that all cylinders were filled to the same level. This top-up varied between about 1-2 kg, i.e. less than 1-2% variation across cylinders. Hence, all the cylinders had a similar bulk density close to 1.35 g cm^{-3}. All cylinders at field capacity weighed between 163 and 165 kg.

The soil in the lysimeters had been fertilized with DAP and muriate of potash, both at a rate of 200 mg kg^{-1} soil. It was also complemented with sieved and sterilized farm manure at a rate of 2:50 to prevent micro-nutrient deficiency. Before growing the sorghum crop, the lysimeters were used for a crop of finger millet and foxtail millet, planted sequentially. The foxtail millet crop had received a urea top-dressing of 3 g $plant^{-1}$. At the end of this crop, only the main root stock from the plants was removed from the top layer of soil by softening the top soil with water and pulling. The soil was then tilled superficially with sickles and limited Alfisol top-up was added so that the surface level was approximately 5 cm below the lysimeter brim. This created a soil profile that was undisturbed from previous cropping, except for minimum tillage of the surface. The lysimeters were then watered to field capacity, based on their expected weight, and the sorghum crop was planted on a full profile. The crop was top dressed with 3 g urea $plant^{-1}$ at 4 weeks after sowing.

150 Space arrangement of the lysimeters and weighing

151 The top of the cylinders was equipped with a metal collar and rings that allowed them to
152 be lifted. Weighing of the cylinders was done by lifting the cylinders with a block-
153 chained pulley, and an S-type load cell (Mettler-Toledo, Geneva, Switzerland) was
154 inserted between the rings of the cylinder and the pulley. The scale (200 kg capacity)
155 allowed repeated measurements with an accuracy of 20 g on each weighing. The
156 lysimeters were separated from one another by a distance of approximately 5 cm. Thus
157 the sorghum crop was planted at a density of approximately 11 plants m^{-2}, a plant
158 population similar to typical field plantings at ICRISAT (row-to-row distance of 60 cm
159 and plant-to-plant spacing of 15 cm). This allowed us to accurately assess the water
160 extraction pattern of a crop cultivated in conditions similar to the field. The tubes were
161 arranged in four trenches of 2 m depth and 1.75 m width. Each trench was separated by a
162 20 cm concrete wall. Possible border effects were expected on the south side of the
163 trenches (these were oriented east-west) and those effects were curbed by bordering the
164 trench with two rows of plants on the south side of the trenches.

165

166 Treatments used and trait assessed

167 Four seeds were planted in each cylinder on 20 October 2008 during the *Rabi* sorghum
168 season. Plants were thinned to two seedlings per cylinder at 14 days after sowing (DAS)
169 and then to one plant per cylinder at 21 DAS. Disturbance to remaining plant was
170 avoided by clipping the thinned plant below the collar. All plants were fully irrigated
171 until 28 DAS. This involved cylinders receiving 500 mL twice a week for the first two
172 weeks after sowing, and then on alternate days until 28 DAS. At 28 DAS, the cylinders
173 were covered with a 2-cm layer of low density polyethylene beads to prevent soil
174 evaporation. Preliminary testing indicated that the beads prevented more than 90% of the
175 soil evaporation, so that differences in mass primarily reflected plant transpiration (data
176 not shown). Biomass increase between weighing was negligible compared to plant water
177 use. Weighing of the cylinders was done at 30 DAS for the first time and then
178 subsequently every two weeks. This gave a total of five weights until harvest for the DS
179 plants and six weights for the WW plants. The first weighing at 30 DAS gave the field

capacity weight of each cylinder. The cylinders were distributed in four trenches and the weighing of one trench per day was done. The same sequence of weighing was used for each trench so that the time intervals between weighing were the same in all cylinders.

To keep the WW plants sufficiently wet for optimum growth and to avoid water drainage after irrigation, the WW plants were watered when the cylinder weight, at the time of weighing, had fallen below 2L from the weight at field capacity. This prevented drainage at the bottom. The watering was done every week. The week that plants were not weighted, the water addition of the previous week was used for watering the WW plants. The DS treatment received no water from 28 DAS until maturity, except for 2L that were added to all cylinders at 73 DAS (beginning of grain filling).

Flowering time (d) was recorded on a plant basis. Transpiration was calculated at approximately 2-weekly intervals between 31 DAS (the time at which weighing started) and maturity. Daily transpiration values were calculated for each plant by dividing the transpiration for each time interval between weighing by the number of days in each interval. Pre-anthesis transpiration was the sum of the daily transpiration values until anthesis, plus water used in the first 28 days after sowing, which was estimated to be 1.5L for all genotypes. This was based on dry biomass estimates of 15 g at 28 DAS and on the assumption of a TE of 10 g kg^{-1} water transpired at such an early stage (our unpublished observations). The post-anthesis water use was the sum of the daily transpiration values from anthesis until maturity. Harvest was done over a period of 4 days. Leaf, stem (including sheath) and panicle masses were recorded after drying for 3 days in a forced-air oven set at 70 ºC. Panicles were then subsequently threshed to determine grain yield. The harvest index was calculated as the ratio of grain yield divided by the total aboveground biomass (the aggregated mass of stems, leaves, and panicles). Transpiration efficiency (TE) was calculated as the ratio of the total aboveground biomass divided by the sum of transpiration values between 30 DAS and maturity. The initial biomass at the time of initiating the transpiration measurements was not taken into account, assuming that biomass differences between genotypes at that stage were negligible. This would have led to a slight over-estimation of TE.

Plant material

The flowering time of 384 lines belonging to the sorghum reference set had been determined under field conditions in 2008-09 (Upadhayaya, pers. comm.). Based on these data, 149 reference set entries and three control cultivars, IS 2205, IS 18758, and IS 33844, varying in flowering time between 70 and 85 days after sowing, were selected. IS 2205 is a Durra-Bicolor landrace resistant to shoot fly and stem borer. IS 18758 is a Guinea-Caudatum landrace, released as E 35-1 in Burkina Faso in 1983 and as Gambella 1107 in Burundi in 1990. IS 33844 is a Durra landrace released in India as Parbhani Moti in 2002. The 149 reference set lines represented 30 out of 44 countries in the entire reference set. Race-wise composition was Caudatum (31), Durra (18), Bicolor (17 accessions), Guinea (14), Kafir (6), Guinea-Caudatum (24), Caudatum-Bicolor (14), Durra-Caudatum (13), Durra-Bicolor (3), Kafir-Bicolor (1), and Kafir-Caudatum (1). An accession each of *aethiopicuum* and *virgatum*, two accessions of *drummondii*, and three of *verticilliflorum* were also part of the 149 reference set material.

In addition to the DS and WW sets of plants used above, a third set of plants was sown at the same time in an area adjacent to the trenches. Plants were grown in 25 cm pots filled with 11 kg of the same Alfisol. Previous experiments in sorghum using these pots showed no signs of growth restriction due to pot size up to anthesis. The same planting procedures were used and plants were kept well-watered until harvest. This set was harvested at flowering and its purpose was to evaluate leaf area and tillering characteristics of the different genotypes at that stage.

Data analysis

The experiment design was an Alpha lattice with 19 blocks of 8 entries within each block. There were three replications and two water regimes (WW and DS). The Residual Maximum Likelihood (ReML) method of Genstat was used to obtain the unbiased estimate of the variance components and the best linear unbiased predictions (BLUPs) for the different parameters measured within each treatment, considering genotypes as random and replications as fixed effects. The significance of the genetic variability among accessions within treatment was assessed from the standard error of the estimate

of genetic variance σ^2_g. Analysis was also performed to assess the effect of genotype (G), water treatment (T) and genotype-by-water treatment (GxT) interaction for the different traits measured. In this case, G and GxT were considered as random effects whereas treatment and replication were considered as fixed effects. The significance of genetic variability across treatments or of the genotype-by-treatment interaction effect was assessed in a manner similar to the above. The significance of the fixed effect of the treatment was assessed using the Wald statistic that asymptotically follows a χ^2 distribution.

For the multi-linear regression analysis, a multi-linear model was used in the software STATA (Stata Corp. College Station, Tx, USA), where yield was taken as an additive function of HI, TE, total water extraction, water extracted in the post-anthesis period, water extracted in the 45-59 DAS and 59-78 DAS period, days to flowering, and a constant. The same multi-linear model was used to assess the residual yield variations not explained by HI (see below), therefore excluding HI from the list of explanatory variables.

Results

Yield and biomass components

Grain yield varied significantly between genotypes under DS conditions, ranging from 0.3 to 36.6 g plant^{-1} (Fig. 1a). Overall, the mean yield of 20.6 g plant^{-1} under DS conditions was about 50% of the yield mean under WW conditions (42.0), indicating that the stress imposed was neither too severe nor too mild (Table 1). Under WW conditions, grain yield varied from 2.1 to 82.8 g plant^{-1}. Grain yield under WW and DS conditions were poorly related (R^2 = 0.10), which also reflected the large genotype-by-treatment (GxT) interaction for grain yield (Table 1).

Harvest index (HI) also varied significantly between genotypes under DS conditions, ranging from 0.05 to 0.52 (Fig. 1b), except for two genotypes that did not produce any grains. The overall mean HI of 0.27 under DS was only slightly smaller than the mean HI under WW conditions (0.33). The HI also varied considerably under WW conditions,

ranging from 0.21 to 0.53, except for five genotypes that had a poor HI lower than 0.15. Contrary to the grain yield data, the HI under DS conditions was better related to the HI under WW conditions (R^2 = 0.33) (Fig. 1b), although HI also displayed a significant genotype-by-water treatment interaction (Table 1). The total plant biomass varied largely between entries. Under DS conditions, there was a two-fold difference between the minimum and the maximum value, whereas under WW conditions these differences were about four-fold. This reflects in part genotypic differences in plant size and tillering, which became larger under WW conditions. Since the genotypes were randomized in the different replications, it is also a possibility that dwarf germplasm may have suffered from shading from tall germplasm in the WW conditions. This possibility is, however, quite unlikely under DS conditions, where the range of total biomass was smaller than under WW conditions, and also where total biomass differences also reflected large differences in grain yield.

Total water extraction

Total water extracted under DS conditions varied significantly ($P<0.001$) among the 149 entries, ranging from 10,600 to 15,200 g plant^{-1}. Noticeably, a low CV of only 6% was obtained for the total water extraction in the lysimetric system. Under fully irrigated conditions, the water extracted by the plants also varied significantly, ranging from 10,500 to 42,300 g plant^{-1}. Besides an expected treatment effect, the total water extracted showed a large GxT interaction effect (Table 2), whereas the genotypic effect was non-significant. In fact, the water extracted under WW and DS conditions showed a poor relationship (R^2 = 0.08). Total water uptake under DS conditions was assessed for each individual race. The Durra race had the highest total water uptake (14,120 g plant^{-1}, n=20) (Fig. 2). The Durra-Caudatum race had, on average, the lowest total water uptake (13,570 g plant^{-1}, n=12), followed by the Caudatum-Bicolor accessions (13,800 g plant^{-1}, n=14).

The first two water use measurements for the 31-45 DAS and 45-59 DAS time intervals were similar in WW and DS plants (Fig. 3), although there was a significant, but minor, treatment effect on the water extraction in the 45-59 DAS interval (Table 2). Indeed, the water used under DS in the 45-59 DAS period was above 70% of that under WW

299 conditions, except for 18 lines where water used was 50-70% of that under WW. This
300 indicated that for the 29 days following the last irrigation in the DS treatment, DS plants
301 extracted similar amounts of water to WW plants. Water uptake in the 59-78 DAS
302 interval showed a large treatment (T) effect on water extraction, and large and significant
303 G and GxT effects, the latter being more important than the G effect. Water uptake in the
304 78-94 DAS interval also showed large T and GxT effects, and no significant G effect. By
305 59 DAS, 125 out of 152 entries had flowered and all the others flowered by 65 DAS.

306 Summarising, the large variation in water extraction capacity under DS conditions, with a
307 tendency to have higher water extraction in Durra race than in Durra-Caudatum race,
308 resulted from specific adaptation of genotypes to the stress conditions, and the temporal
309 pattern of water use indicated that stress occurred after flowering for most lines.

310

311 *Relationships between water extracted before and after anthesis*

312 The pre-anthesis water use varied by 9 L plant^{-1} among genotypes (5 to 14 L plant^{-1}
313 range). These differences were, in part, explained by the flowering time (R^2 = 0.70, data
314 not shown) although large variations in pre-anthesis water use per day, which removes
315 the differences due to flowering time, remained (101 to 205 g water per day). Pre-
316 anthesis water use was also significantly correlated to the leaf area at anthesis (R^2 = 0.18,
317 data not shown). Pre-anthesis water use under DS was also predominantly determined by
318 genetic effects (Table 2). The post-anthesis water used ranged from about 2 to 10 L
319 plant^{-1} among genotypes. Post-anthesis water use under DS was correlated to flowering
320 time (R^2 = 0.73) but not to the post-anthesis water use of WW plants. Pre- and post-
321 anthesis water use showed a close negative correlation (R^2 = 0.83) (Fig. 4). Post-anthesis
322 water use was also predominantly determined by genotype-by-treatment interaction
323 effects, whereas the G effects were not significant (Table 2). Post-anthesis water use was
324 also negatively correlated to the leaf area at anthesis (R^2 = 0.17). These data indicate that
325 despite flowering time determining about two thirds of the variation in pre- and post-
326 anthesis water use, there was still a large range of variation in pre- and post-anthesis
327 water use at any level of flowering time.

328

Transpiration efficiency

Transpiration efficiency varied largely among entries, ranging from 3.21 to 6.09 g kg^{-1} water transpired under DS conditions. The coefficient of variation was only 13.6%. Under fully irrigated conditions, TE also varied significantly, ranging from 2.95 and 5.59 g kg^{-1} (Table 1). The grand mean of 4.30 g kg^{-1} was lower than under DS conditions (4.82 g kg^{-1}). TE under DS and WW conditions were correlated but the correlation coeficient was weak (R^2 = 0.13, data not shown). Besides a strong treatment effect on TE, G and GxT interaction effects were both significant although the magnitude of the Ge effects was slightly higher. Transpiration efficiency was assessed for each individual race under DS conditions. The Guinea race exhibited the lowest mean TE values (4.29g kg^{-1}, n=13), followed by the Kafir (4.58g kg^{-1}, n=6), whereas the Guinea-Caudatum, Durra and Caudatum races had the highest mean TE values (5.09, 5.05 and 4.98 g kg^{-1}, n = 25, 20 and 32, respectively) (Fig. 5). In summary, TE was mostly driven by genotypic effects rather than by GxT interactions, and high TE variants were identified, especially in the Guinea-Caudatum, Durra and Caudatum races.

Relationships between water extraction, TE, HI, and yield

Regression analyses were conducted between grain yield and WU, TE, and HI. The relationship between grain yield and water used was significant under fully irrigated conditions only (R^2 = 0.33), but not under DS conditions (data not shown). Similarly, grain yield was significantly related to TE under WW conditions (R^2 = 0.35) and, although the relationship was significant under DS conditions, the correlation coefficient was weak (R^2 = 0.07) (data not shown).

Therefore, individually, neither the total water used nor TE had any substantial bearing on yield under DS conditions. This was because the relationship between yield and HI was highly significant, and more so under DS conditions (R^2 = 0.88) than under WW conditions (R^2 = 0.53) (Fig. 6a). However, for any given HI level, Figure 6a indicates clearly that substantial variation in yield remained unexplained by HI, especially at HI levels above 0.30. These residual yield variations unexplained by HI were calculated by subtracting the yield predicted by the regression equation (Fig. 6a) from the observed

yields, following Vadez et al (2007b). These residuals showed a highly significant correlation with TE ($R^2 = 0.60$) (Fig. 6b) and the total water extracted ($R^2 = 0.40$; data not shown). Similarly, for any given TE level, yield variation remained unexplained by HI and TE. These residual yields were calculated in a similar way, using the regression equation of Fig 6b. These residual yields, unexplained by either the HI or TE, were closely related to the total water used ($R^2 = 0.43$ or 0.35, excluding or including an outlier data point on the left of the regression curve) (Fig. 6c). Other regressions were tested between these residuals and the pre- and post-anthesis water use, and the pre- and post-anthesis water used normalized by the flowering time, but no significant relationships were found (data not shown).

Similar results were observed from a multi-linear regression, where an additive model was used (Table 3), among several others that were tested. The model explained 98% of the phenotypic variation in yield. In that analysis, HI was the explanatory variable accounting for the largest component of yield, followed by TE and water used. In this model, the post-anthesis water use had a significant negative effect on yield, while the water extracted in the 59-78 DAS period, corresponding to the flowering stage of most genotypes, had a significant positive effect on yield. Finally, the time to flowering had a significant negative effect on grain yield (Table 3). The same approach was used to explain the residual yield variations not explained by HI. The best model explained 79% of the variation in the residuals and parameters having the most influence were TE and the total water use, with TE having a probability coefficient about twice that of total water use (Table 4). In that model, the water extracted in the 45-59 DAS and 59-78 DAS intervals were both significantly and positively related to the residual yield variations.

In summary, besides a strong HI effect on yield under DS conditions, the large yield variations remaining unexplained by HI were mostly driven by TE, and then next by total water extraction differences.

Discussion

Our data showed a two-fold range of variation for TE and almost 20% variation for water extraction (3L $plant^{-1}$, equivalent to 30 mm on a field basis) under water stress in this

389 selection of lines from the sorghum reference collection. Water extraction and TE
390 measurements in the lysimeters were reliable, exhibiting very low CVs (6% and 13.6%
391 ,respectively). After removing the proportion of yield differences explained by HI, there
392 was still a substantial yield variation unexplained, and this was highly significantly
393 related to TE. Water extraction only ranked third when accounting for yield variations
394 unexplained by either HI or TE. High genetic variation for TE and water extraction offer
395 substantial breeding opportunities, with high TE and water extraction variants in the
396 Durra race being a critical source of key terminal drought adaptation traits for sorghum.
397 Importantly, high TE and water extraction are not necessarily mutually exclusive, as
398 evidenced by variants of the Durra race.

399

400 Large genotypic differences in TE

401 Transpiration efficiency was increased by about 10% under DS compared with WW
402 conditions and since the GxT interactions were rather small, differences were driven by
403 genotypic effects. This contrasted with previous results reporting a higher TE under fully
404 irrigated conditions (Donatelli *et al.* 1992). Also the range of variation reported here
405 under both water regimes was almost two-fold higher than in previous work reporting
406 20% (Donatelli *et al.* 1992) and 25% (Hammer *et al.* 1997) differences in TE. The values
407 reported here were slightly below the range found by Balota et al (2008) (5.04 – 7.55 g
408 kg^{-1} water) which is the only reported study where TE was measured over the entire crop
409 cycle. The values here were also slightly below that in Hammer et al (1997) (6.0 – 7.7 g
410 kg^{-1}). Many of these differences could simply be related to differences in VPD under
411 different conditions. Also roots were not included in our TE assessment (inclusion of
412 roots could increase absolute TE values by about 30-40%). There was no significant
413 relationship between TE and total water extraction, which could be used as a proxy for
414 root mass. This led us to conclude that the non inclusion of root mass in our TE
415 calculation was unlikely to undermine much of the genetic differences in TE explained
416 here.

417 In the current work, TE was highly correlated with total biomass (R^2 = 0.82, data not
418 shown), which is similar to that found recently by Xin *et al.* (2009), but different from

Hammer *et al.* (1997). Also, the absence of relationship between TE and total water use, tested here in a large and representative set of germplasm, undermines previous speculation that TE and water use could be negatively related (Blum 2005) and clearly shows that it is possible to find germplasm capable of exhibiting both high water extraction and high TE, as previously shown (Peng and Krieg 1992). Indeed, both high water extraction and high TE were exhibited by variants of the Durra race in this study. When comparing races, it appeared that both Caudatum and Durra had overall higher values of TE than other landraces. Therefore, large genotypic variation for TE, especially in Caudatum and Durra races, could be exploited by sorghum breeding programs. The reasons for the superiority of the Caudatum and Durra races is unknown but we speculate that some could be in the environmental conditions in which these landraces have evolved. It has been recently shown that certain sorghum genotypes restrict transpiration at high vapor pressure deficit (Ghoolipoor et al., 2010), which could lead to water saving and/or differences in transpiration efficiency.

Large differences in water extraction capacity

Little work has been done to assess water extraction *per se*, except for a detailed study on two genotypes by Robertson et al. (1993). Indeed, most studies in different crops so far have relied on assessing rooting characteristics and not on the function of root systems (Siddique *et al.* 1990; Sanguinetti *et al.* 1998; Kashiwagi *et al.* 2005). Therefore, our study may be the first to report a large range in variation for water extraction across an extensive germplasm set. Because of the lack of a strong relationship between water use under WW and DS conditions, water extraction differences were not related to constitutive traits but rather to differences in a response to stress. The low coefficient of variation (6%) for total water extraction measurements clearly indicates the value of the lysimetric system for assessing water extraction. In addition, it resolves previous complications related to identifying drought-adaptive rooting traits (Price *et al.* 2002).

These water extraction differences could relate to either a deeper rooting (Singh et al., 2010), or to differences in the effective depth of water extraction. We estimate that each cylinder contains a total of 27 L of water (about 23% w/w is a typical value for this

449 Alfisol), from which about 70% can be extracted for transpiration (unpublished data).
450 Therefore, approximately 19 L of water would then be available for extraction from the
451 soil profile of the lysimeters, assuming the root length density was sufficient to do so.
452 Hence, the sorghum genotypes extracting the most water from the profile, i.e. about 13 L
453 after deducting 2L of water that were added in the course of the experiment, had roots
454 sufficient to extract all possible water from about 70% of the soil profile. By contrast,
455 genotypes extracting the least water would have attained full extraction to only about
456 50% of the soil profile. For the sake of representing these water extraction differences in
457 terms of rooting depth, a 3 L $plant^{-1}$ difference represents all the water available in a 40
458 cm depth of the lysimeter soil profile.

459 Our method of assessing the pattern of water extraction during the whole life cycle is an
460 innovation that adds precision and high throughput to existing field-based equipments
461 (e.g. TDR, neutron probes). Rather than relying on static and destructive measurements
462 of rooting characteristics, lysimeters sown with individual plants enabled water use to be
463 evaluated under controlled water regimes in a dynamic manner. It revealed a large range
464 of variation in both pre- and post-anthesis water use, and that these two parameters were
465 highly and negatively correlated. Although both pre- and post-anthesis water use were
466 related to flowering time, Figure 4 also clearly shows that for a similar level of pre-
467 anthesis water use, there was still variation in post-anthesis water use, indicating that
468 genotypes varied in their capacity to extract water during the grain filling period. More
469 work is needed to assess how post-anthesis water use affects yield in genotypes with
470 similar flowering time and pre-anthesis water use, as previously considered (Hammer
471 2006). The profile of water extraction also provided insight into when sorghum plants are
472 getting stressed, i.e. not until about 4 weeks after the last irrigation. Although this type of
473 information has been acquired from the field (Ritchie, 1981; Steiner, 1983, Turner et al.
474 1986), it is possible to acquire at a much larger scale and with more precision using the
475 lysimetric system, enabling detailed studies of the relationships between patterns of water
476 use and grain yield.

477

478 Besides HI, yield relates closely to TE and third only to water use

Yield variations under DS had no relation with the yield potential (Fig. 1). With a crop density of 11 plant m^{-2} in the lysimetric system, the mean trial grain yield under terminal stress corresponded to 2.1 t ha^{-1}, which was in the range of yield previously observed in the field with post-rainy sorghum materials of 1.2 – 2.1 t ha^{-1} under similar weather conditions (Mahalakshmi and Bidinger 2002). Yields under fully irrigated conditions corresponded to 4.2 t ha^{-1}, indicating that the stress imposed in the cylinders was within the desired range.

Among the components of Passioura's equation, HI explained the largest portion of the genetic variation in yield. This would be expected from germplasm with such a large range of variation for HI under both water regimes, and also from the fact that yield and HI have terms in common. Nevertheless, Figure 6 also showed that yield variation remained unexplained by HI. Our multi-linear approach, using a similar methodology to that in previous work (Bidinger *et al.* 1987; Vadez *et al.* 2007b), shows that the effect of HI on the yield variation needs to be removed before the strong effect of TE on the substantial residual variations can be highlighted. This reveals the important impact of TE on yield differences under terminal stress conditions in sorghum, as previously suggested (Hammer *et al.* 1997; Sinclair *et al.* 2005; Xin *et al.* 2009).

Interestingly, under well-watered conditions, the harvest index had much less of an influence on yield than under terminal stress (R^2 = 0.57 vs R^2 = 0.88). By contrast, both TE and water use (WU) had a significant effect on yield (R^2 = 0.34 for TE; R^2 = 0.16 for WU) when water was not limiting. In that case, we also computed the residual yield unexplained by HI. The correlation between these residuals and TE was also highly significant (R^2 = 0.29, $P < 0.01$), although it did not improve the direct relationship that was previously found between TE and yield. By contrast, the residuals were highly correlated to the water used (R^2 = 0.69), with a significant improvement compared to the relationship previously drawn between WU and yield. These data then support the importance of maximizing water use when water is non-limiting, as previously suggested (Blum 2005).

These data highlight the potential of the lysimetric system to accurately assess the various components of Passioura's equation, allowing the respective weighting of their

509 importance under different watering regimes. Work is now in progress to test a number of
510 water deficit conditions to assess the possible change in the bearing of each component.
511 In addition, other traits also had a significant bearing on yield under terminal drought. In
512 particular, extracting more water during the 59-78 DAS time interval, which
513 corresponded to flowering and early grain filling, had a significant and positive effect on
514 yield. This was despite the highly variable germplasm used in this work (germplasm with
515 differences in tillering and harvest index). These data suggest that water uptake during
516 that period may be critical for successful seed setting and grain filling of the crop, as
517 previously shown and suggested (Ratnakumar *et al.* 2009; Vadez *et al.* 2007a; Zaman-
518 Allah et al., 2011), and also found in pearl millet (Vadez *et al.* 2009).

519 Summarising, the lysimetric system was suitable for a) generating yield data that
520 approximate field conditions, and b) assessing the value of the various components of the
521 Passioura equation on yield. The use of either step-wise regression or multi-linear
522 regression analysis was needed to remove components of greater influence in order to
523 highlight the significant influence of other traits.

524

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531

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668

Table 1. Trial means, range of expected means, standard error of differences (SED) within treatment, and wald statistics and F-probability for genotype effect (G), treatment effect (T) and genotype-by-treatment (GxT) interaction related to time to flowering (d), grain dry mass (g plant^{-1}), total dry mass (g plant^{-1}), harvest index (HI), transpiration efficiency (TE, g kg^{-1}) and panicle harvest index (PNHI, i.e. the ratio of the grain weight by the panicle weight)

		50% Fl		Grain yield		Total DW		Harvest index		TE		PNHI	
		WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
Mean		57	56	41.97	20.59	126.94	67.05	0.33	0.27	4.38	4.82	0.75	0.70
Min		46	44	2.06	0.23	34.01	41.75	0.02	0.00	2.95	3.21	0.26	0.00
Max		67	72	82.83	36.76	193.29	88.47	0.53	0.45	5.59	6.09	0.89	0.89
σ^2_g		13.1	17.6	166.0	38.69	664.6	43.47	0.06173	0.00584	0.162	0.162	0.005493	0.00623
SE		1.9	2.3	28.2	6.53	106.3	8.89	0.00098	0.00095	0.054	0.038	0.00099	0.00118
SED		2.14	2.02	10.07	4.80	18.83	5.99	0.057	0.056	0.45	0.39	0.061	0.067
G	σ^2_g	15.0		41.6		113.2		0.00496		0.113		0.005677	
	SE	1.94		12.5		40.7		0.00080		0.032		0.000942	
T	Wald	14.6		325		731.6		56.5		78.4		33.1	
	Prob	0.001		0.001		0.001		0.001		0.01		0.001	
G x T	σ^2_{gxT}	0.65		61.1		236.7		0.001342		0.049		0.001065	
	SE	0.42		12.4		43.7		0.000433		0.032		0.000540	

Table 2. Trial means, range of expected means, standard error of differences (SED) within treatment, and wald statistics and F-probability for genotype effect (G), treatment effect (T) and genotype-by-treatment (GxT) interaction related to total water use (g plant^{-1}), pre-anthesis water use (g plant^{-1}), post anthesis water use (g plant^{-1}), and water used in the 45-59 DAS (g plant^{-1} d^{-1}), 59-78 DAS (g plant^{-1} d^{-1}) and 78-94 DAS (g plant^{-1} d^{-1}) periods

Water use		Total		Pre-anthesis		Post-anthesis		45-59 DAS		59-78 DAS		78-94 DAS	
		WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
Mean		28,856	13,908	9,383	8,956	20,727	6,452	358	344	401	180	368	112
Min		10,500	10,620	5,406	4,493	5,854	2,439	141	162	114	70	99	58
Max		42,320	15,240	15,493	13,628	35,018	10,917	556	668	598	279	630	229
σ^2_g		27,908,726	158,959	3,641,052	1,731,958	22,464,015	1,982,010	4,528	1,452	4,482	677	7,954	334
SE		4,141,939	50,275	535,809	285,982	3,435,089	301,436	759	969	838	260	1,164	76.7
SED		3,467	436	1,147	999	3,053	961	52	49	57	30	57	18
G	σ^2_g	1,124,515		2,183,060		1,452,707		2641		974		75	
	SE	1,456,941		328,263		1,238,507		600		375		430	
T	Wald	1,017		8.95		1,132		4.19		702		953	
	Prob	0.001		0.003		0.001		0.04		0.001		0.001	
G x T	σ^2_{gxT}	12,814,903		365,709		10,488,093		247		3509		4187	
	SE	1,939,986		144,457		1,572,411		53.9		641		620	

Table 3. Multi-linear regression between yield and several explanatory variables under DS conditions: harvest index, transpiration efficiency, total water extracted, post-anthesis water use, water used in the 45-59 DAS and 59-78 DAS periods, and days to flowering

Factors	Coefficient	SE	t-value	P > t
Harvest index	67.6	0.77	87.73	0.000
Transpiration efficiency	4.28	0.14	29.59	0.000
Total water extracted	0.00163	0.00015	10.90	0.000
Post-anthesis water use	-0.00038	0.00017	-2.30	0.023
Water use between 45-59 DAS	0.00011	0.00015	1.81	Ns
Water use between 59-78 DAS	0.00045	0.00019	2.32	0.021
Days to 50% flowering	-0.161	0.053	-3.02	0.003
Constant	-34.30	3.92	-8.74	0.000

Table 4. Multi-linear regression between the residual yield variations not explained by HI and several explanatory variables under DS conditions: transpiration efficiency, total water extracted, post-anthesis water use, water used in the 45-59 DAS and 59-78 DAS periods, and days to flowering

Factors	Coefficient	SE	t-value	P > t
Transpiration efficiency	3.44	0.18	18.49	0.000
Total water extracted	0.00147	0.00019	7.77	0.000
Post-anthesis water use	-0.00044	0.00022	-2.02	0.046
Water use between 45-59 DAS	0.00029	0.00012	2.39	0.018
Water use between 59-78 DAS	0.00066	0.00025	2.64	0.009
Days to 50% flowering	-0.105	0.069	-1.53	ns
Constant	-32.12	5.11	-6.28	0.000

Figure Captions

Fig. 1. Relationship between grain yield under terminal water stress (g plant^{-1}) and grain yield under well-watered conditions (g plant^{-1}) (a), and relationship between harvest index (HI) under terminal water stress and HI under well-watered conditions (b) in 152 germplasm entries. Data are the mean of 3 replicated lysimeter-grown plants per genotype.

Fig. 2. Total water extracted from the lysimeter soil profile (g plant^{-1}) under terminal water stress conditions in the different sorghum races. Data are the mean of the average transpiration values within each race (Bicolor, n=17; Caudatum, n=31; Caudatum-Bicolor (C-B), n=14; Durra, n=18; Durra-Caudatum (D-C), n=13; Guinea, n=14; Guinea-Caudatum (G-C), n=24; Kafir, n=6).

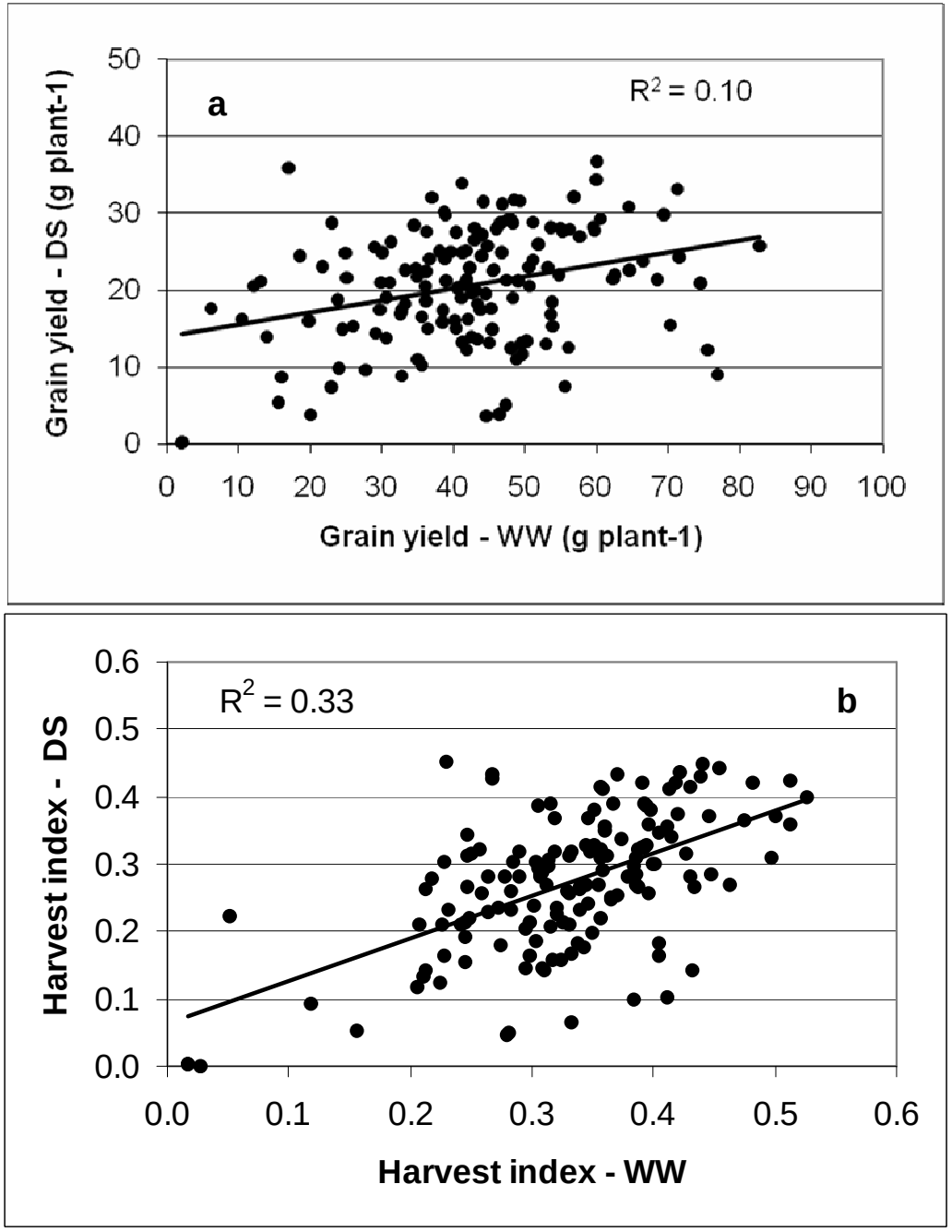
Fig. 3. Profile of transpiration (g plant^{-1}) as a function of time after sowing. Last irrigation was applied at 30 DAS in plants exposed to terminal water stress (DS, open symbols) and well-watered conditions (WW, closed symbols). Data are the mean (± SE) of the average transpiration values for 152 germplasm entries. For the DS and WW plants, five and six lysimeter weighings, respectively, were done, giving four and five transpiration intervals.

Fig. 4. Relationship between the pre-anthesis water use (g plant^{-1}) and the post-anthesis water use (g plant^{-1}) in152 germplasm entries. Data are the mean of 3 replicated lysimeter-grown plants per genotype.

Fig. 5. Transpiration efficiency (g kg^{-1} water transpired) under terminal water stress conditions in the different sorghum races. Data are the mean of the average transpiration values within each race (Bicolor, n=17; Caudatum, n=31; Caudatum-Bicolor (C-B), n=14; Durra, n=18; Durra-Caudatum (D-C), n=13; Guinea, n=14; Guinea-Caudatum (G-C), n=24; Kafir, n=6).

Fig. 6. Relationship between (a) seed yield under terminal water stress and the harvest index (HI), (b) relationship between the residual yield variations unexplained by HI and transpiration efficiency (TE), and (c) relationship between the residual yield variations unexplained by either HI or TE and the total water extracted from the soil profile in 152 germplasm entries. Data are the mean of 3 replicated lysimeter-grown plants per genotype.

Figure 1


a
R^2 = 0.10
Grain yield - DS (g plant-1)
Grain yield - WW (g plant-1)
0
10
20
30
40
50
60
70
80
90
100
b
R^2 = 0.33
Harvest index - DS
Harvest index - WW
0.0
0.1
0.2
0.3
0.4
0.5
0.6

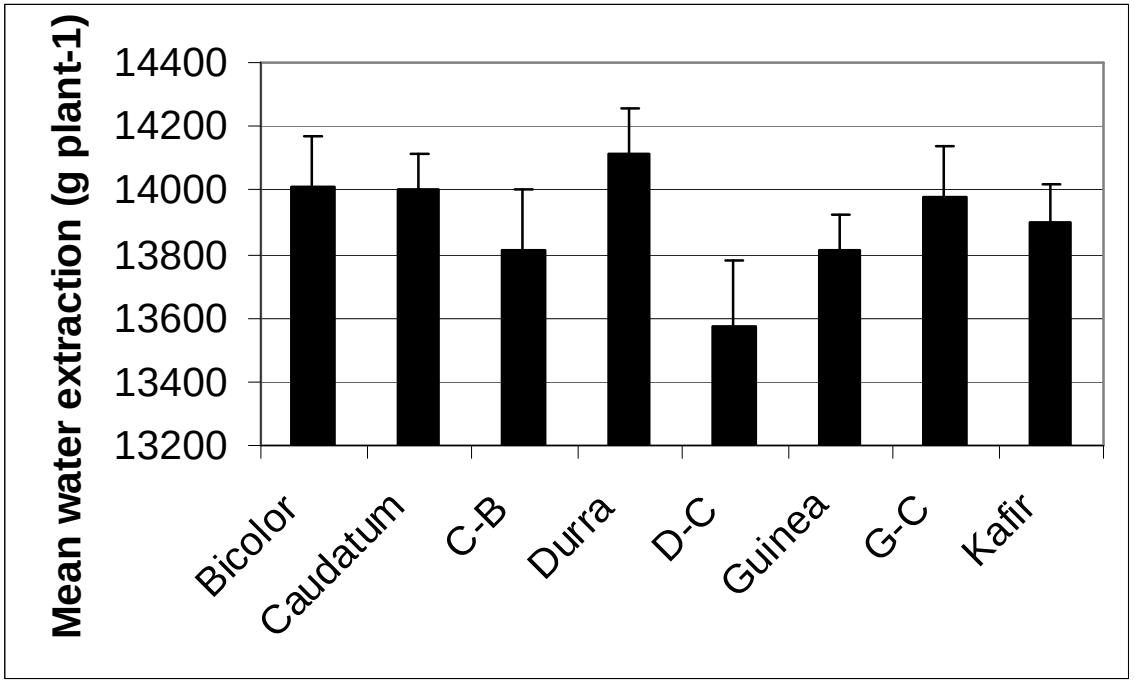

Mean water extraction (g plant-1)
14400
14200
14000
13800
13600
13400
13200
Bicolor
Caudatum
C-B
Durra
D-C
Guinea
G-C
Kafir

Figure 2

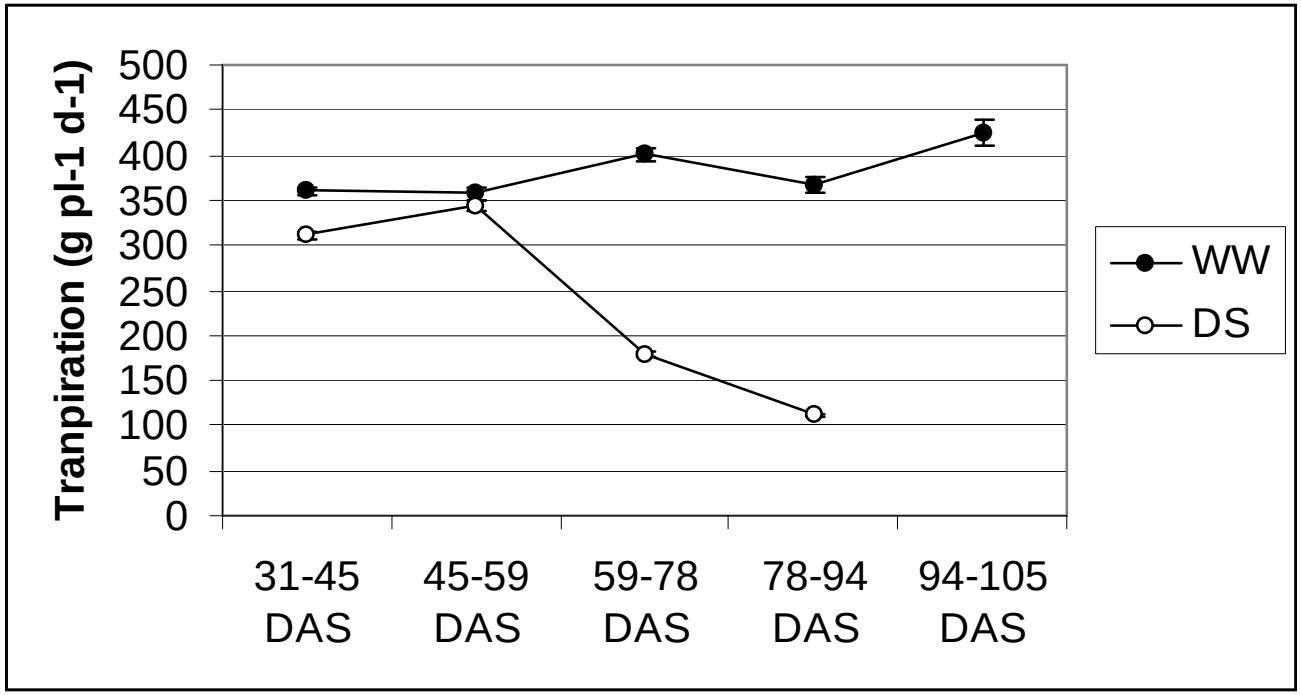

Tranpiration (g pl-1 d-1)
500
450
400
350
300
250
200
150
100
50
0
31-45 DAS
45-59 DAS
59-78 DAS
78-94 DAS
94-105 DAS
WW
DS

Figure 3

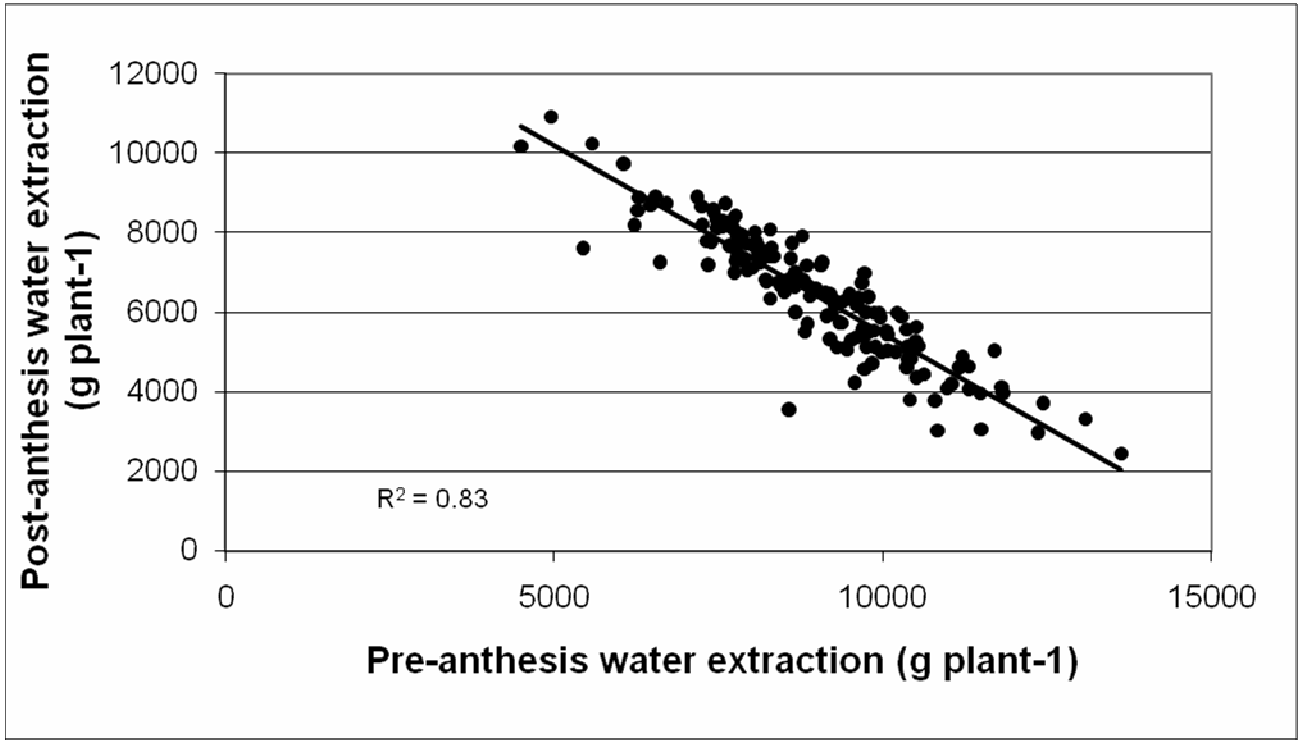

Post-anthesis water extraction (g plant-1)
12000
10000
8000
6000
4000
2000
0
R2 = 0.83
0
5000
10000
15000
Pre-anthesis water extraction (g plant-1)

Figure 4

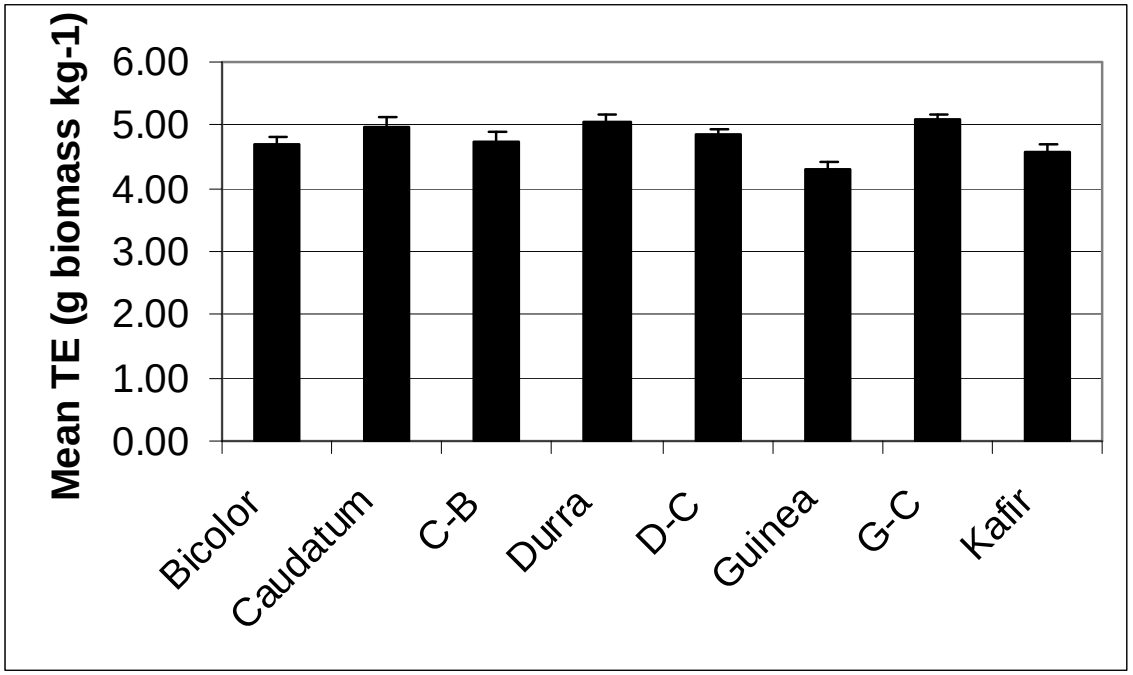

Mean TE (g biomass kg-1)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Bicolor
Caudatum
C-B
Durra
D-C
Guinea
G-C
Kafir

Figure 5

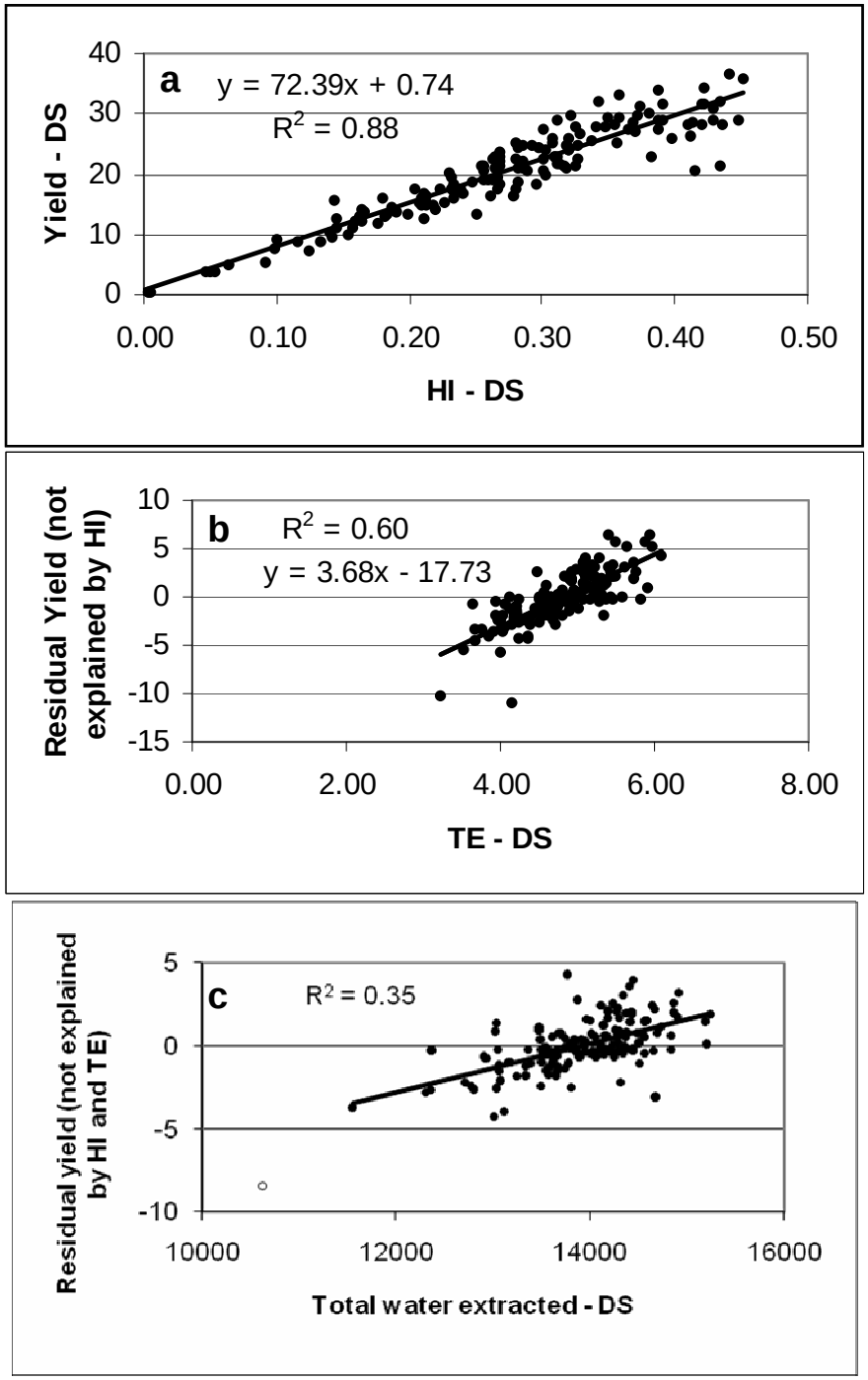

a
y = 72.39x + 0.74
R2 = 0.88
Yield - DS
HI - DS
0
10
20
30
40
0.00
0.10
0.20
0.30
0.40
0.50
b
R2 = 0.60
y = 3.68x - 17.73
Residual Yield (not explained by HI)
TE - DS
10
5
0
-5
-10
-15
0.00
2.00
4.00
6.00
8.00
c
R2 = 0.35
Residual yield (not explained by HI and TE)
Total water extracted - DS
5
0
-5
-10
10000
12000
14000
16000

Figure 6